

Effect of the acute and chronic administration of *Lupinus albus* β -conglutinin on glycaemia, circulating cholesterol, and genes potentially involved

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ARTICLE INFO

Keywords:

Legumes

Nutraceuticals

Postprandial hyperglycemia

Glucose metabolism

Anticholesteremic agents

Gene expression profiling

ABSTRACT

Constituents of lupin seeds, like γ -conglutinin and lupanine, have gained attention as potential complementary treatments for dysglycaemia management. Notwithstanding, the effect of other lupin components on carbohydrate metabolism, including β -conglutinin protein, has received little attention. Here, we investigated the influence of the acute and chronic administration of β -conglutinin on glycaemia modulation in normal and streptozotocin induced-to-diabetes rats. We analysed the liver transcriptome modulation exerted by β -conglutinin in diabetes-induced rats using DNA microarrays to scout for potential molecular targets and pathways involved in this biological response. The acute administration of β -conglutinin reduced the incremental area under the curve of glycaemia in normal and diabetes-induced animals. In a seven-day study with diabetic animals, glycaemia increased significantly in non-treated animals but remained unchanged in animals treated with a daily dose of β -conglutinin. Total cholesterol was significantly lower at the end of the experimental period (-21.8% , $p = 0.039$). The microarray and gene ontology analyses revealed several targets and pathways potentially modulated by β -conglutinin treatment, including a possible down-regulation of Jun kinase activity. Moreover, our data indicate that targets related to oxidative stress, inflammation, and estrogenic activity might orchestrate these metabolic effects. In conclusion, our findings show that β -conglutinin may help manage postprandial glycaemia and reduce cholesterol levels under the dysglycaemia stage. We identified and proposed new potential molecular targets for further research related to the mechanism of action of β -conglutinin.

1. Introduction

Food and nutrients' role in preventing and managing non-communicable diseases is widely recognized and of current scientific interest. Consumption of unprocessed and processed meat is associated with a higher risk of cancer, coronary heart disease, stroke, and type 2 diabetes (T2D), while plant-based diets exhibit an inverse relationship with the risk of developing these diseases [1]. Basic and clinical research results indicate that besides being a protein source, legume seed consumption has beneficial health effects. For instance, legumes promote weight loss and reduce the risk of metabolic syndrome [2–4]. Similarly, experiments with animal models have shown that the consumption of diverse legumes and their constituents has positive results on glucose and lipid metabolism, inflammation, and oxidative stress [5].

In particular, the consumption of high-quality foods such as vegetables, fruits, nuts, whole grains, and legumes can aid in the prevention and management of T2D [6]. More specifically, the consumption of lupin seeds and their proteins could improve human health by modulating lipid and glucose metabolism [7].

Lupin is a Leguminosae distributed worldwide and consumed since

ancestral times in various world regions. It includes wild and domesticated species, with seeds rich in protein and low to high alkaloid levels. *Lupinus albus*, a high protein-low alkaloid domesticated species, is commonly incorporated into the human diet in the Mediterranean area [8]. Various studies indicate that the consumption of lupin seeds and its proteins, specifically γ -conglutinin, positively affects glucose metabolism. Related to this, results by our research group and others suggest that γ -conglutinin exhibits insulin-mimetic activity and reduces hepatic glucose production [9–11]. Lupin proteins consist of two main classes, albumins, and globulins, with an approximate quantity ratio of 1:9. The lupin globulin proteins include four fractions: α -, β -, γ -, and δ -conglutinins [12]. Although most of the studies related to lupin proteins' hypoglycaemic effects focus on γ -conglutinin, it is plausible that the other fractions may influence glycaemia to some extent. Despite this, we do not know much about the bioactivity of other lupin protein fractions on glucose homeostasis.

Recently, using recombinant β -conglutinins, a modulatory effect was observed on targets related to inflammation and insulin signalling cascade [13,14]. Moreover, β -conglutinin peptides derived from its *in vitro* digestion exhibited inhibitory activity on dipeptidyl peptidase IV (DPP-IV) [15,16], which is known to play a prominent role for insulin secretion by limiting the secretagogue activity of incretin

Abbreviations: BW, body weight; DEG, differentially expressed genes; DPP-IV, dipeptidyl peptidase IV; GO, gene ontology; iAUC, incremental area under the curve; OGTT, oral glucose tolerance test; OSTT, oral starch tolerance test; STZ, streptozotocin; T2D, type 2 diabetes.

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<https://doi.org/10.1016/j.biopha.2020.110969>

Received 7 September 2020; Received in revised form 29 October 2020; Accepted 1 November 2020

Available online 7 November 2020

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hormones. Thus, we aimed to assess the *in vivo* effects of the acute and chronic administration of native *L.albus* β -conglutinin on glycaemia. We evaluated the former by measuring the blood glucose response in normal and induced-to-diabetes rats after a β -conglutinin acute oral dose. For the latter, we determined the fasting serum biochemical parameters after diabetic rats consumed a β -conglutinin oral daily dose for seven days. Considering that liver function is pivotal for glucose homeostasis, we evaluated the transcriptome modulation induced in this tissue by β -conglutinin treatment in animals induced to diabetes as a first exploratory approach to propose potentially involved genes. We hypothesised that the acute and chronic administration of β -conglutinin reduces glycaemia levels in a murine model and modifies the expression profile of liver genes related to carbohydrate metabolism.

2. Materials and methods

2.1. Chemicals and drugs

J.T.Baker (Avantor, Inc., PA, USA) supplied analytical grade sodium chloride, ammonium sulphate, sodium phosphate, sodium acetate, and acetic acid. Acarbose (commercial, for clinical use) and sterile saline were from Laboratorios PISA (Jalisco, Mexico), dextrose from Analytika (Nuevo Leon, Mexico), and we purchased corn starch from a local food distributor (Jalisco, Mexico). Streptozotocin and rosiglitazone ($\geq 98\%$ HPLC) were acquired from Sigma-Aldrich (MO, USA) and Zoletil 100 from Virbac (Carros, France). BioSystems (Barcelona, Spain) provided us with the reagents required for determining the serum biochemical parameters.

2.2. Plant material and β -conglutinin isolation

Certified *L. albus* seeds, kindly supplied by Dr. Edzard van Santen, were dehulled, ground into flour, and hexane-defatted. β -conglutinin protein was isolated from flour following the method described by Martinez-Ayala & Paredes-Lopez (2001) [17]. In brief, lupin flour was dispersed in double-distilled water (DDW) at a 1:10 flour-to-water ratio (w/v) and stirred for 2 h at 4 °C. Next, we centrifuged the suspension for 30 min at $10,370 \times g$ and 4 °C (hereafter, referred to as centrifugation or centrifuged) and discarded the supernatant. We suspended the globulin-containing pellet in 10 % NaCl solution (pH 7), gently stirred for 12 h at 4 °C, and centrifuged. Then, we precipitated the globulins from the supernatant by 85 % ammonium sulphate saturation and centrifugation. Next, we dissolved the pellet in 0.1 M phosphate buffer (pH 6.8) and dialyzed against 0.2 M acetate buffer (pH 4.8) for 18 h, and centrifuged. We discarded the α -conglutinin containing pellet and dialyzed the β - and γ -conglutinin rich supernatant against DDW for 48 h at 4 °C. Finally, we centrifuged the solution and lyophilized the β -conglutinin containing pellet for 8 h at $-50\text{ }^{\circ}\text{C}$ and 0.036 mbar pressure in a Freeze Drying 4.5 equipment (LABCONCO, MO, USA).

2.3. Characterization of isolated β -conglutinin

The lyophilized β -conglutinin fraction was characterized by denaturing sodium dodecyl sulphate-polyacrylamide electrophoresis (SDS-PAGE). Four mg-samples from each lyophilized β -conglutinin fraction were dissolved in Laemmli buffer, heated to 90 °C for 5 min in a water bath, and centrifuged 10 min at $9725 \times g$ and 4 °C. The supernatants were recovered and underwent SDS-PAGE (12 % polyacrylamide) in a Mini-Protean Tetra Cell (Bio-Rad, Milan, Italy). After electrophoretic separation, the gels were stained with G-250 Coomassie blue (Bio-Rad, Milan, Italy), and the β -conglutinin fraction characterized by molecular weight comparison against a BenchMark™ Prestained protein ladder (Fig. A.1 in supplementary material; Invitrogen, Thermo Fisher Scientific, CA, USA). For every 100 g of defatted flour, we recovered 3.09 g of β -conglutinin. Considering that previous studies with γ -conglutinin indicate a glycaemia modulatory effect, we performed a Western blot analysis using a commercial antibody against γ -conglutinin (Agrisera, Vannäs, Sweden) to rule out the presence of this protein in the enriched β -conglutinin fraction (Fig. A.2 in supplementary material).

2.4. Animals

Male Wistar rats weighing 200 – 250 g, purchased from the Universidad de Guadalajara Bioterium, were housed in an air-conditioned room ($24 \pm 2\text{ }^{\circ}\text{C}$, $55.0 \pm 5\%$ relative humidity) with a 12 h light/dark cycle starting at 07:00 h. Unless specified otherwise, rats had free access to water and a standard rodent chow diet (LabDiet, PMI Nutrition International, MO, USA). The experimental procedures complied with the International Guidelines for the Care and Use of Laboratory Animals and the Mexican Official Standard 062 (NOM-062-ZOO-1999). Experiments were designed and performed according to the Three Rs tenet (Replacement, Reduction, and Refinement), adhering to animal experimentation's ethical liabilities. The Universidad de Guadalajara Bioethics Committee reviewed and approved the experimental protocol (registration number: C.I./023/2014).

2.5. Experimental protocol in normal animals

In this study, we evaluated how the acute administration of lupin β -conglutinin affected normal rats' glycaemic response. We used a within-subject experimental design with animals and both the oral glucose tolerance test (OGTT) and the oral starch tolerance test (OSTT). In the OGTT, normal animals received three treatments each: saline (vehicle) and β -conglutinin at two doses (120 or 240 mg/kg of body weight (BW) in saline). To explore aspects of carbohydrate digestion, we decided to assess the effect of β -conglutinin on the absorption of complex carbohydrates using the OSTT. In this case, normal rats also received three treatments: saline (vehicle), acarbose (30 mg/kg BW, in saline), or β -conglutinin (120 mg/kg BW, in saline). Test solutions were administered by oral gavage with a wash-out period of at least three days between treatments.

2.5.1. Oral glucose tolerance test

Although maintenance of glucose homeostasis involves complex modulatory machinery, the OGTT is a useful screening technique for evaluating how food

components and pharmacological compounds may affect glucose metabolism [18]. Thirty min before an oral glucose load (2 g/kg BW), we force-fed the test solutions to overnight (12 h)-fasted rats ($n = 3$). We then drew blood by vein tail puncture at time 0 (immediately before the oral glucose load) and every 30 min for 120 min. The blood glucose level was determined using a glucometer (One Touch Ultra®, Johnson & Johnson, NJ, USA) and plotted against time. We calculated the positive incremental area under the curve (iAUC) for each animal using the trapezoid rule.

2.5.2. Oral starch tolerance test

Normal rats ($n = 3$) were fasted for 12 h and force-fed the trial solutions (saline, acarbose in saline, or β -conglutinin in saline). One hour later, we drew a blood sample (time 0) and orally administered a starch load (2 g/kg BW). Glycaemia levels were subsequently monitored with a glucometer (One Touch Ultra®, Johnson & Johnson, NJ, USA) at one-hour intervals for four hours in blood collected by vein tail puncture. We graphed the blood glucose level vs. time and calculated the iAUC for each animal using the trapezoidal rule.

2.6. Evaluation of the effects of β -conglutinin on animals induced to diabetes

This study also measured the glycaemic effect of acute and chronic administration of β -conglutinin on rats induced to diabetes by streptozotocin (STZ). The STZ-induced rat model of diabetes is a widely used and accepted method to study potential antidiabetic therapies [19].

2.6.1. Diabetes induction by STZ

Twelve hour-fasted animals were induced to diabetes with a single intraperitoneal STZ injection (65 mg/kg BW). Before its use, we prepared the STZ solution in ice-cold 0.1 M acetate buffer (pH 4.5). After the induction procedure (72 h), we determined the blood glucose concentrations in fasted animals and included in the experiments those exhibiting glycaemia ≥ 200 mg/dl.

2.6.2. Glycaemia response to acute and chronic administration of β -conglutinin

The influence of two acute β -conglutinin doses (120 or 240 mg/kg BW) or saline on the glycaemia of STZ-induced animals ($n = 6$) was monitored as described for non-diabetic animals.

We assessed the effect of the chronic intake of β -conglutinin by measuring the changes in seven biochemical blood parameters at the end of a seven-day trial. Twenty-four rats were randomly allocated into the following four groups (six rats per group): 1) **Ctrl**, non-diabetic animals without treatment (only vehicle); 2) **STZ-Ctrl**, STZ-induced diabetic rats without treatment (only vehicle); 3) **STZ- β C120**, animals induced to diabetes with STZ and treated with 120 mg β -conglutinin/kg BW; 4) **STZ-RSG**, STZ-induced diabetic animals treated with 3 mg rosiglitazone/kg BW. On the first day of the trial, we collected blood samples from the retro-orbital plexus of rats fasted for 12 h and anesthetized with Zoletil 100 (tiletamine-zolazepam, 80 mg/kg BW). During the next seven days, animals received a single dose of the vehicle (saline) or treatment by oral gavage at 09:00 a.m., and on the 8th day, we drew blood

specimens from fasted, anesthetized animals. Blood samples were allowed to clot; the serum was separated by centrifugation ($2580 \times g$ for 15 min at 4°C) and stored at -80°C until the determination of pre- and post-treatment biochemical parameters.

2.6.3. Determination of serum biochemical parameters

Serum parameters (glucose, urea, creatinine, triglycerides, total cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST)) before and after treatment were determined with a semi-automatic BTS-350 spectrophotometer and commercial reagents (BioSystems, Barcelona, Spain).

2.7. RNA isolation from liver tissue

We isolated and purified RNA from the liver tissue of treated and untreated STZ induced-to-diabetes animals to analyse the hepatic gene expression profile. After seven days of treatment with saline or β -conglutinin (120 mg/kg BW), the liver was excised from anesthetized animals, washed in sterile saline, and homogenized in RLT buffer. Next, we isolated total RNA with the RNeasy Mini Kit (QIAGEN, Thermo Fisher Scientific, CA, USA) following the manufacturer instructions and stored the RNA samples at -80°C until use. Before the microarray assays, we tested the RNA quality and integrity by agarose electrophoresis (1%) and determined its concentration and purity using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, CA, USA).

2.8. Printing of DNA microarrays

The liver transcriptome from diabetic animals treated with β -conglutinin was compared against that of untreated animals using a two-colour DNA microarray. The existing rat DNA microarray is not comprehensive; however, there is a high homology between rat and mouse genomes. Thus, to broaden our capabilities of screening gene expression changes, we decided to employ a mouse microarray covering all genes as performed by other researchers [20].

The arrays were printed with a mouse 65-mer oligo library containing 22,000 gene-specific oligonucleotide probes (Sigma-Genosys, TX, USA). Using 25×75 mm SuperAmine coated slides (TeleChem International Inc., CA, USA), 50 μM Oligo library suspended in Micro Spotting solution (ArrayIt Brand Products, CA, USA) was used to print the arrays fixating at 80°C for 4 h. Afterward, the slides were water vapor-rehydrated at 60°C and the probes fixed with two UV light-cycles (1200 J). Next, the slides were maintained at 92°C for 2 min, washed with 95 % ethanol for 1 min and pre-hybridized in 5X SSC, 0.1 % SDS, and 1% BSA at 42°C for 1 h. Finally, the slides were washed and dried for subsequent hybridization.

2.9. Labelled cDNA synthesis and array hybridization

We obtained labelled cDNA by reverse transcription of purified mRNA using dUTP-Alexa555 or dUTP-Alexa647 fluorophores with the First-Strand cDNA Synthesis kit (Thermo Fisher Scientific, CA, USA). We determined the fluorophore markers'

concentration incorporated into cDNA by recording the absorbance at 555 nm and 650 nm for Alexa555 and Alexa647, respectively. Next, equal amounts of labelled cDNA were hybridized into the arrays with UniHyb solution (TeleChem International Inc., CA, USA) for 14 h, 42 °C.

To decrease overall biological variability [21], we carried out the cDNA synthesis by combining equivalent amounts of high-quality RNA from the individual animal in each group into a single pool. We separately pooled high-quality RNA from the treated (with β -conglutin, $n = 6$) and non-treated (without β -conglutin, $n = 6$) groups and carried out the cDNA synthesis and hybridization. cDNA synthesis was performed using the fluorescent dUTP nucleotides separately. After hybridization, images from the arrays were acquired and analysed.

2.10. Microarray data acquisition

The microarray images were acquired and quantified using a GenePix 4100-A reader with GenePix software (Molecular Devices, CA, USA). The mean density fluorescence of Alexa555 and Alexa647, background, and signal cross channel Loess normalization (by subgrids) was performed for each spot with the ArrayPro Analyzer software (Media Cybernetics Inc., MD, USA).

The genArise software was employed (Computing Unit of Cellular Physiology Institute of UNAM, <http://www.ifc.unam.mx/genarise/>) to analyse the microarray data. The software package allows the data analysis of two-colour microarrays and records the differentially expressed genes (DEG) by calculating an intensity-dependent Z-score. The genArise software corrects the background values, carries out a Lowess normalization, filter by intensity, eliminates low-quality measurements, and adjusts the fluorescence intensities to facilitate comparisons. For each data point, the mean and standard deviation were calculated using a sliding window algorithm to determine the Z-score, where Z measures the standard deviations a data point is from the mean. For this first exploratory approach, we included elements with a $|Z\text{-score}| > 1.50$ for further bioinformatics analysis. The calculation of the Z-score values was performed with the formula below.

$$Z_i = (R_i - \text{mean}(R)) / \text{sd}(R)$$

Where: Z_i , Z-score for each element; R_i , the log-ratio for each element; $\text{sd}(R)$, the standard deviation of the log-ratio.

Gene ontology (GO) analyses of DEG were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 [22,23]. Enriched terms regarding the molecular function, biological processes, and cellular location of DEG were identified. The up-regulated or down-regulated DEG were separately submitted to the platform. Modified Fisher Exact (EASE score) p values < 0.05 were established as threshold values to identify enriched GO terms in the distinct categories. To visualize the network interactions of DEG modulated by β -conglutin, we used STRING and the Cytoscape software [24,25]. The microarray results

were deposited in the Gene Expression Omnibus database (GSE156579).

2.12. Statistical analysis

We report the acute and chronic trials' results as the mean $\pm$ standard error of the mean (SEM). To calculate the iAUC, we subtracted the baseline glucose value from each subsequent reading before constructing the curve and computed the positive area. The glycaemia curves and iAUC data from acute experiments were analysed by two- or one-way within-subjects ANOVA followed by Holm-Sidak *post hoc* to assess differences among treatments. The analyses were performed using GraphPad Prism 8.0.1 for Windows (GraphPad Software, CA, USA). We used a two-tailed paired t -test to compare the pre- vs. post-treatment serum biochemical parameters for the chronic treatments. We considered as statistically significant those p values < 0.05 .

3. Results

3.1. β -conglutin improves oral glucose tolerance in normal rats

We used the OGTT to assess the effect of two acute doses (120 and 240 mg/kg BW) of β -conglutin on normal rats' glycaemic response. The acute administration of β -conglutin (120 or 240 mg/kg BW), 30 min before the glucose load, attenuated the glycaemic response compared to the Ctrl saline solution (Fig. 1A-B). As seen in both figures, β -conglutin significantly lowered the glycaemia response. After the initial peak, blood glucose levels decreased faster in animals treated with β -conglutin than in the control group (Ctrl), indicating a higher blood glucose clearance rate. As shown in Fig. 1C, the iAUC for animals treated with β -conglutin was significantly smaller ($p < 0.05$) than that of the saline Ctrl group: -54% and -59% , for the 120 and 240 mg/kg BW groups, respectively; however, there was no difference in the glycaemic response for the β -conglutin doses ($p = 0.68$).

3.2. Effect of β -conglutin on the digestion and absorption of starch

Since there was no difference in the glycaemic response of the two β -conglutin doses in the OGTT experiments, we assessed the 120 mg/kg BW dose effect in the OSTT. Fig. 2 includes the results of the OSTT in normal rats. Acute administration of β -conglutin shifted the glycaemia response curves downward between the acarbose and Ctrl (saline) groups (Fig. 2A-B). Remarkably, its iAUC was significantly lower than that of the Ctrl group ($3,050.3 \pm 654.9$ vs. $5,416.0 \pm 1,337.2$, $p < 0.05$). Likewise, the administration of acarbose attenuated the glycaemic response significantly as compared to Ctrl ($2,336.4 \pm 848.1$, $p < 0.05$), whereas the acarbose and β -conglutin groups had similar responses ($p = 0.28$) (Fig. 2C). The glycaemia peak (at 60 min) for rats treated with acarbose was substantially lower than that of animals treated with vehicle ($p < 0.001$) or β -conglutin ($p < 0.05$). Moreover, administering β -conglutin lowered the glycaemia at 120 min compared to the Ctrl

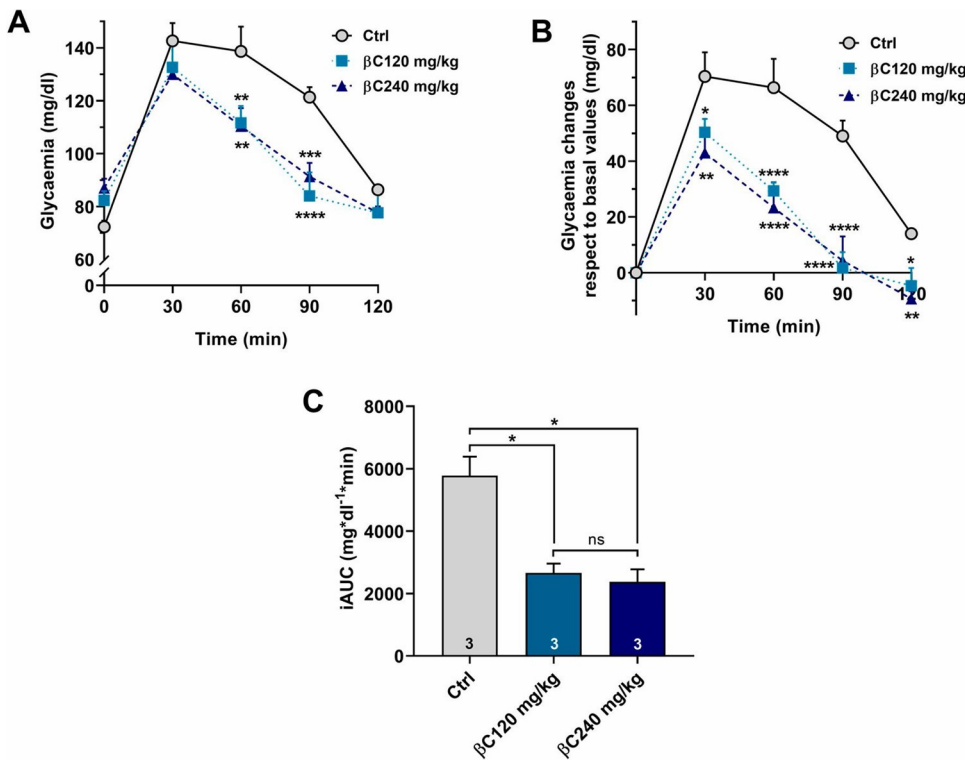
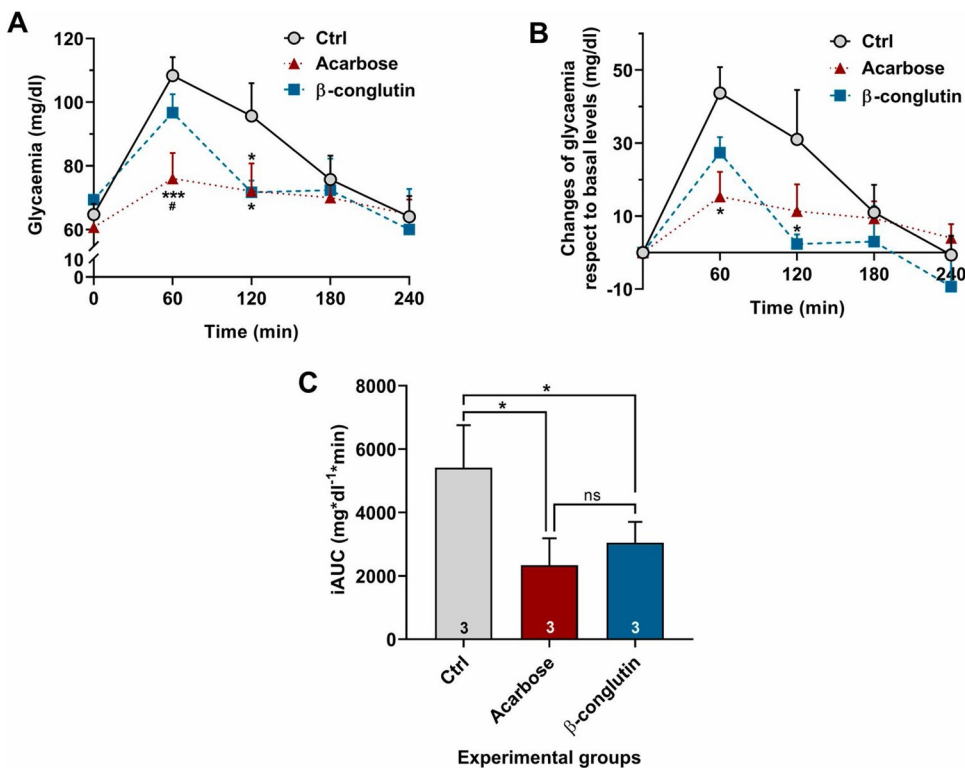


Fig. 1. Oral glucose tolerance test in normal rats treated with *L. albus* β -conglutin. Adult Wistar rats were fasted overnight (12 h) and orally treated without (Ctrl, only

Holm-Sidak *post hoc* test. Numbers inside bars indicate the *n* for each condition. ns, not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



area under the curve (iAUC) was calculated using the trapezoid rule. Statistical analysis included two- and one-way within-subjects ANOVA and Holm-Sidak *post hoc* test. Numbers inside bars indicate the *n* for each condition. ns, not significant; * $p < 0.05$, *** $p < 0.001$ vs. Ctrl; # $p < 0.05$ acarbose vs. β -conglutin.

vehicle) or with β -conglutin (β C120 or β C240 mg/kg BW) 30 min before administering an oral glucose load (2 g/kg BW). (A) Blood glycaemia was measured immediately before the glucose load ($t = 0$ min) and monitored for 120 min at 30 min intervals. (B) Before calculating the incremental area under the curve (iAUC), we subtracted the glucose baseline values from each subsequent measurement and graphed the values vs. time. (C) The iAUC of the Ctrl group was significantly larger than that of the β -conglutin groups. Data were analysed using two- or one-way within-subjects ANOVA and

Fig. 2. Oral starch tolerance test in normal rats treated with *L. albus* β -conglutin. After a 12 h fast and 60 min before a starch load, we force-fed Wistar rats a saline (Ctrl), β -conglutin-saline (β -conglutin; 120 mg/kg BW), or acarbose-saline treatment (acarbose; 30 mg/kg BW). (A) Glycaemia levels were determined just before the starch load ($t = 0$ min) and at 60 min intervals for 240 min, and values plotted vs. time. (B) We normalized blood glucose concentrations considering baseline values as zero and graphed the glucose changes over time. (C) The incremental

group ($p < 0.05$).

3.3. Effect of the acute β -conglutin treatment on hyperglycaemia of STZ diabetes-induced rats

The glycaemia levels in STZ diabetes-induced rats treated with two acute β -conglutin doses changed little over time, resulting in relatively

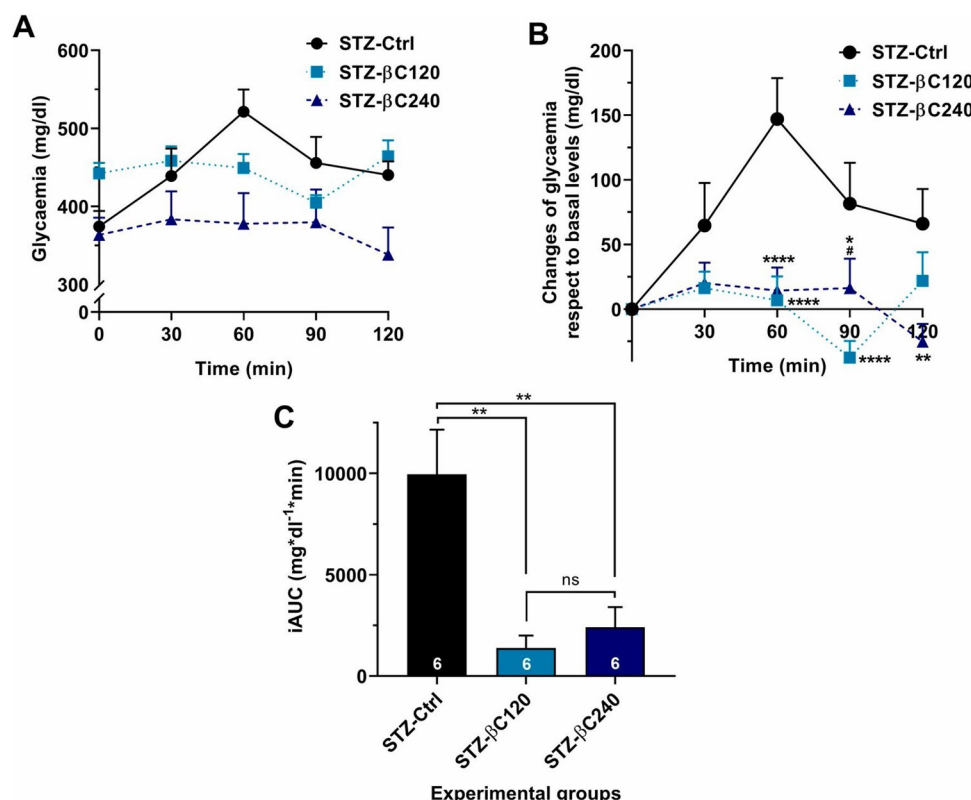


Fig. 3. Glycaemic response to the acute administration of *L. albus* β -conglutin on rats induced to diabetes with STZ. Wistar rats were fasted overnight (12 h) and orally treated with either saline (STZ-Ctrl), β -conglutin 120 mg/kg (STZ- β C120), or β -conglutin 240 mg/kg BW (STZ- β C240). (A) Blood glucose levels were measured immediately before administering the treatment ($t = 0$ min) and every 30 min to 120 min. (B) To construct the incremental area under the curve (iAUC), the baseline glycaemia was subtracted from every successive reading, and (C) the incremental area calculated using the trapezoid rule.

Statistical analysis of the data included two- or one-way within-subjects ANOVA and Holm-Sidak *post hoc* test. Numbers inside bars indicate the n for each condition. (B): * $p < 0.05$, ** $p < 0.01$, **** $p < 0.001$ β -conglutin vs. Ctrl; # $p < 0.05$ β -conglutin 240 vs. 120 mg/kg. (C): ns, not significant; ** $p < 0.01$.

flat curves (Fig. 3A); however, the baseline glycaemia levels were different in all three treatments. To adjust for the differences in the initial glycaemic status, we subtracted the glucose level at time zero from all subsequent readings (Fig. 3B) and constructed the iAUC, resulting in contrasting incremental areas (Fig. 3C). Animals treated with β -conglutin had significantly lower iAUC, but there was no difference between the two protein doses in STZ-induced rats.

3.4. Seven-day β -conglutin treatment does not influence fasting hyperglycaemia but decreases total serum cholesterol levels of induced-to-diabetes rats

We assessed the effect of chronic administration of β -conglutin by comparing the serum levels of seven biochemical parameters before and after treatment (Table 1). In the Ctrl group, the biochemical parameters remained unchanged after the seven-day trial. In contrast, STZ-Ctrl rats exhibited fasting hyperglycaemia at the start of the trial (pre-treatment), and the serum glucose levels increased significantly during the seven-day trial ($p = 0.003$). There were no differences between the pre- and post-treatment levels of the other six biochemical parameters. In STZ diabetes-induced rats, the administration of β -conglutin (120 mg/kg BW) positively affected glycaemia; fasting glycaemia

levels did not change significantly after the seven-day trial in these rats ($p = 0.45$). A novel finding was that the STZ- β C120 group had significantly lower total cholesterol levels after the chronic administration of β -conglutin (-21.8% , $p = 0.039$). We did not observe changes in the other biochemical parameters of STZ- β C120 animals. Furthermore, rosiglitazone decreased serum glucose and triglyceride levels but increased total cholesterol in STZ-RSG rats.

3.5. Analyses of the liver gene expression profile of induced-to-diabetes animals treated with β -conglutin

To explore the potential molecular mechanisms on which the observed biological effects could rely, we analysed the liver transcriptome of STZ induced-to-diabetes animals chronically treated with β -conglutin. Although the results obtained from microarray assays are usually not considered conclusive [26], the data can provide an insight into the genes and pathways involved in a biological response at a specific time and condition and are useful to establish the basis for planning and conducting additional studies that will help to clarify the role of β -conglutin in glucose and cholesterol metabolism at the molecular level. We identified 821 down-regulated and 1603 up-regulated

hepatic genes ($|Z\text{-score}| > 1.5$) in STZ diabetes-induced animals treated with β -conglutin as compared to untreated rats (Fig. 4).

Once the differentially expressed genes (DEG) were identified in the β -conglutin treated group, we performed a gene ontology analysis of these DEG, using the DAVID software, and documented the main cellular compartments, biological processes, and molecular functions related to these genes. **Table 1**

3.5.1. Cellular compartments

After β -conglutin treatment, the ontological analysis (GO) of down-regulated genes showed an enrichment of 28 cellular compartment terms (**Table A.1** in supplementary material). Membrane (33.9 %), cytoplasm (32.2 %), and extracellular exosome (15.1 %) were the terms including most of the analysed genes. On the other hand, the β -conglutin up-regulated genes showed an enrichment of 43 terms (**Table A.2** in supplementary material). In this case, the cytoplasm (31.2 %), nucleus (28.7 %), and membrane (30.6 %) terms included the highest proportion of the genes. Fig. 5 includes the top ten cellular compartments terms enriched by DEG after chronic administration of β -conglutin.

3.5.2. Biological processes implicated in the β -conglutin treatment

The biological processes enriched by the DEG after β -conglutin treatment were also analysed. From the down-regulated genes, we identified 61 different biological process-related terms (**Table A.3** in supplementary material). The regulation of transcription (11.8 %), transcription (10.6 %), and transport (9.7 %) terms comprised most of the down-regulated genes. By significance of enrichment, we identified a down-regulation of Jun kinase in the 11th position (Fig. 6). In contrast, we found that 87

biological process terms were associated with the genes up-regulated by the chronic administration of β -conglutin (**Table A.4** in supplementary material). Most of these genes were included in the transcription (9.4 %), positive regulation of transcription (6.5 %), and transport (8.9 %) terms. Fig. 6 contains the terms with the highest significance of enrichment.

3.5.3. Molecular functions affected by the β -conglutin treatment

By GO analysis of DEG, we also identified possible molecular functions that may be involved in the response of diabetes-induced rats to β -conglutin. The down-regulated genes enriched 19 different terms (**Table A.5** in supplementary material). The predominant molecular function terms included protein binding (22.8 %), transcription factor activity (5.8 %), and sequence-specific DNA binding (4.3 %).

In contrast, β -conglutin up-regulated genes enriched 34 terms (**Table A.6** in supplementary material). Out of these terms, protein binding (21.6 %), nucleotide binding (9.5 %), and protein homodimerization activity (4.6 %) were the most predominant. Fig. 7 contains the top ten molecular function-related terms with the highest enrichment significance.

3.6. Network of genes related to metabolic processes potentially influenced by β -conglutin treatment

A visual representation of the genes possibly involved in the metabolic response to chronic intake of β -conglutin by induced-to-diabetes animals, as well as the interrelationship network among them, was generated through Cytoscape (Fig. 8). In **Table 2**, we show the Z-score values of down-regulated and up-regulated genes included in the analysis considering their possible metabolic implication. In addition, we

Parameter	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx	Pre-Tx	Post-Tx
Glucose (mg/dl)	99.5 $\pm$ 8.8	98.3 $\pm$ 13.8	480.5 $\pm$ 34.7	627.1 $\pm$ 36.0**	329.0 $\pm$ 77.3	395.5 $\pm$ 41.7	496.4 $\pm$ 20.5	393.4 $\pm$ 23.6*
Urea (mg/dl)	50.5 $\pm$ 4.1	41.8 $\pm$ 2.6	56.0 $\pm$ 2.8	61.4 $\pm$ 3.1	46.0 $\pm$ 3.5	58.3 $\pm$ 4.4	50.0 $\pm$ 2.7	66.7 $\pm$ 2.1
Creatinine (mg/dl)	0.74 $\pm$ 0.02	0.86 $\pm$ 0.04	0.84 $\pm$ 0.05	0.92 $\pm$ 0.06	0.75 $\pm$ 0.03	0.78 $\pm$ 0.03	0.94 $\pm$ 0.1	0.88 $\pm$ 0.1
Cholesterol (mg/dl)	60.3 $\pm$ 6.0	45.7 $\pm$ 5.3	64.0 $\pm$ 3.6	72.1 $\pm$ 8.7	67.8 $\pm$ 4.5	53.0 $\pm$ 3.8*	52.9 $\pm$ 6.1	69.6 $\pm$ 5.5*
Triglycerides (mg/dl)	37.2 $\pm$ 10.7	40.6 $\pm$ 3.6	40.8 $\pm$ 8.0	40.2 $\pm$ 7.4	20.0 $\pm$ 2.3	22.8 $\pm$ 3.8	37.5 $\pm$ 5.6	20.8 $\pm$ 3.5*
AST (IU/L)	80.0 $\pm$ 11.6	90.5 $\pm$ 3.7	70.0 $\pm$ 3.42	87.5 $\pm$ 5.2	77.0 $\pm$ 9.8	71.7 $\pm$ 3.8	78.4 $\pm$ 9.2	83.0 $\pm$ 9.0
ALT (IU/L)	77.6 $\pm$ 3.6	70.7 $\pm$ 4.8	56.3 $\pm$ 4.2	69.2 $\pm$ 4.3	65.7 $\pm$ 7.2	66.0 $\pm$ 12.6	63.3 $\pm$ 2.6	60.5 $\pm$ 4.0

Fasting biochemical analytes in the serum of animals induced to diabetes by streptozotocin and treated with β -conglutin (120

	Ctrl	STZ-Ctrl	STZ- β C120	STZ-RSG
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mg/kg BW) for seven consecutive days.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ctrl, non-induced control animals; STZ-Ctrl, streptozotocin-induced animals treated with vehicle; STZ- β C120, streptozotocin-induced animals treated with β -conglutin (120 mg/kg BW); STZ-RSG, streptozotocin-induced animals treated with rosiglitazone (3 mg/kg BW). Data are presented as mean $\pm$ SEM. Two-tailed paired Student's *t*-test was used to evaluate differences between pre-treatment (Pre-Tx) and post-treatment (Post-Tx) mean values. * *p* < 0.05; ** *p* < 0.01.

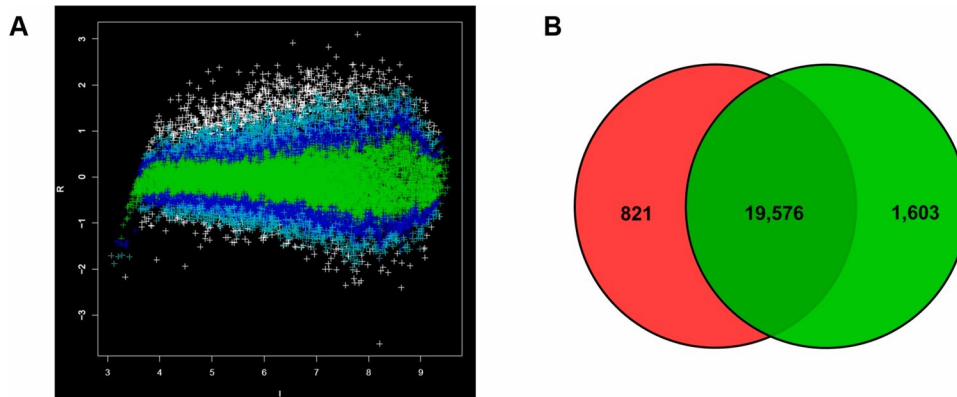


Fig. 4. Liver gene expression of induced-to-diabetes rats treated with β -conglutin. Wistar rats were induced to diabetes by streptozotocin and treated without (vehicle) or with β -conglutin (120 mg/kg BW) for seven

consecutive days. At the end of the experimental period, liver mRNA was isolated and reversed- transcribed into labelled cDNA. Using a dual- colour microarray, we identified the differentially expressed genes (DEG) by comparing the transcript levels from diabetic animals treated with β -conglutin vs. those of untreated rats. (A) Log-ratio vs. intensity plot of the gene expression in liver samples from induced-to-diabetes animals treated with β -conglutin. DEG with a $|Z\text{-score}| > 1.5$ is presented in light blue and white colour. (B) Venn diagram summarizes differentially down-regulated (red/left circle) and up-regulated (green/right circle) genes identified by the genArise software. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

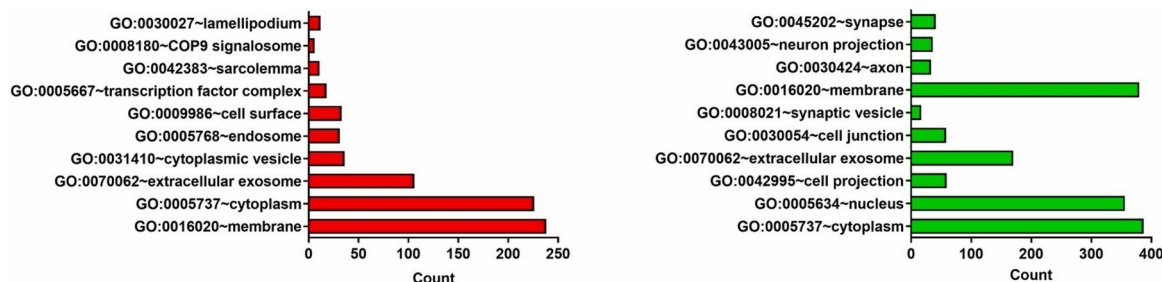


Fig. 5. Cellular compartment terms enriched by differentially expressed genes in liver tissue of streptozotocin-induced rats treated with *L. albus* β -conglutin. Gene expression profile was determined using a dual-colour DNA microarray. Enriched terms were identified using DAVID. The count of down-regulated (red bars, left section) or up-regulated (green bars, right section) genes included in each term is shown on the X-axis. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

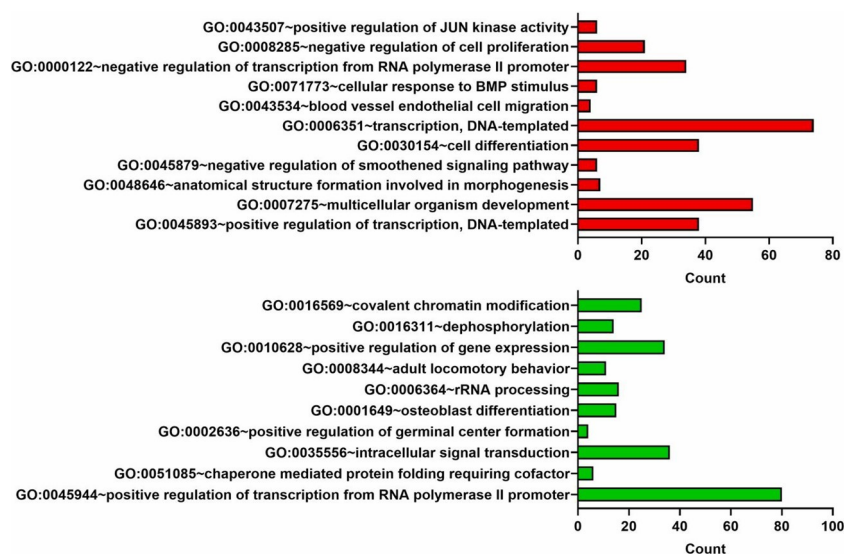


Fig. 6. Biological processes enriched by potentially modulated genes in hepatic tissue of streptozotocin-induced-to-diabetes rats treated with *L. albus* β -conglutin. Enriched terms were identified using DAVID. The abscissa represents the count of down-regulated (red bars, upper section) or up-regulated (green bars, lower section) genes included in each term. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

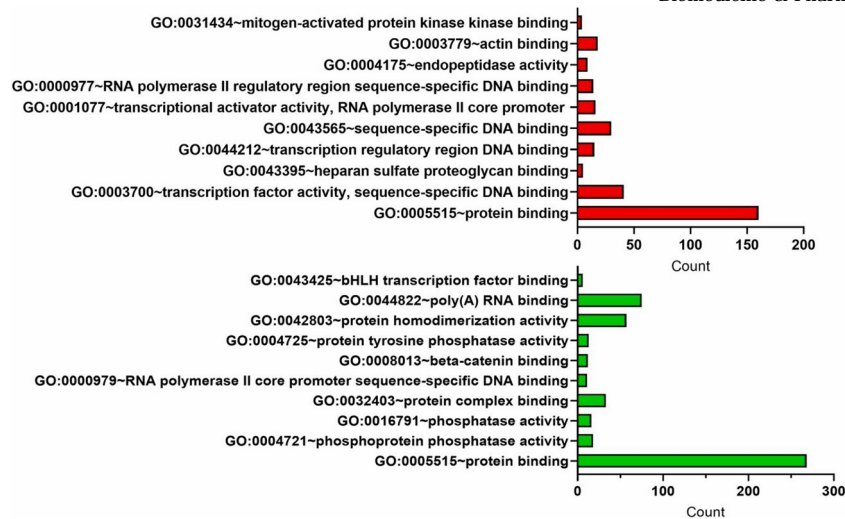


Fig. 7. Molecular function terms enriched by differentially expressed genes in the liver tissue of rats induced to diabetes and treated with *L. albus* β -conglutinin. Enriched terms were identified using DAVID. The X-axis represents the count of down-regulated (red bars, upper section) or up-regulated (green bars, lower section) genes included in each term. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

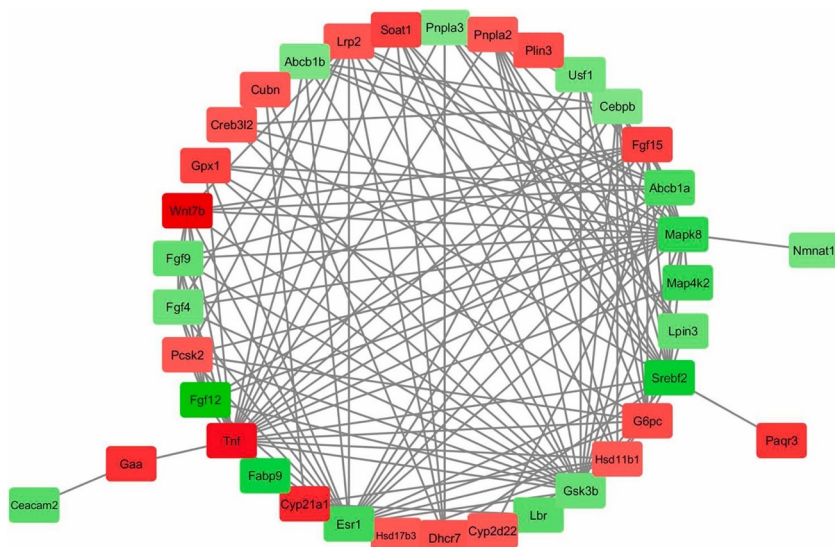


Fig. 8. Network interaction of differentially expressed genes in the liver of induced-to-diabetes rats chronically fed *L. albus* β -conglutinin. The differentially expressed genes related to the metabolism were selected, and their interaction documented using STRING and analysed by Cytoscape, including the Z-score values derived from the microarray experiments. Colour intensity is proportional to the down-regulation (red boxes) or up-regulation (green boxes) recorded for each gene after β -conglutinin treatment. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

identified the hub genes potentially involved in the effects of *L. albus* β -conglutinin (Fig. 9). In these networks, different molecular targets can be observed, including the up-regulated *Gsk3b*, *Src*, *Kras*, *Esr1*, *Srebf2*, and the down-regulated *Tnf*, *Hras*, *Ptpcr*, *Pnpla2*, *Gpx1*, and *Fgf15* genes. **4. Discussion**

The study of complementary therapies for the management of hyperglycaemia is of utmost importance in public health. Understanding functional foods and nutraceuticals' role in preventing and ameliorating dysglycemia, diabetes, and associated complications has increasingly gained scientific interest. Different studies have reported the beneficial effects of diverse legumes on glucose homeostasis, including soybean, fenugreek, beans, adzuki beans, and yellow peas, among others [27–31]. In the case of lupins, studies focused on the effect of some lupin-derived products, such as protein isolate hydrolysate, lupin hydroethanolic extracts, or specific protein fractions like γ -conglutinin,

have suggested a favourable influence on glucose homeostasis [32–34]. To expand our knowledge of the effects of lupin constituents on glucose metabolism, we evaluated the effect of acute and chronic administration of β -conglutinin, a major lupin protein fraction, on postprandial and fasting glycaemia modulation.

Our experiments in normal rats revealed that acute doses of β -conglutinin attenuated the glycaemic response in the OGTT significantly. Similarly, our data showed a beneficial effect on hyperglycaemia of induced-to-diabetes animals that received an acute protein dose. The lupin β -conglutinin is a 7S protein homologous to soybean β -conglycinin. In agreement with our results, Tachibana et al. reported that normal rats fed β -conglycinin exhibited a lower glycaemic response and higher insulin sensitivity than animals fed casein [35]. Thus, it is tempting to speculate that homologous legume proteins may induce a similar, beneficial influence on glycaemia.

Despite the positive results obtained with lupin β -conglutinin, its action mechanism needs to be clarified. This protein might slow down the digestion and absorption of complex carbohydrates or influence

other glucose metabolic processes at the systemic level. To test the former, we evaluated the influence of the protein fraction on OSTT. Our data showed that β -conglutinin iAUC was significantly lower than that of control saline and was not different from the iAUC of acarbose treatment. However, the administration of β -conglutinin promoted a rapid

Table 2

Metabolism-related genes potentially influenced by β -conglutinin treatment (120 mg/kg BW) in rats induced to diabetes by streptozotocin.

Up-regulated genes			Down-regulated genes				
Accession number	Gene symbol	Z-score value	Accession number	Gene symbol	Z-score value		
NM_009480							
Usf1	1.619131	NM_008061	G6pc	-	1.675129		
NM_009883	Cebpb	1.511026	AK018174	Paqr3	- 1.88264		
NM_007543	Ceacam2	1.858175	NM_008064	Gaa	- 1.910915		
AJ272163	Esr1	2.035011	NM_013693	Tnf	- 2.137131		
NM_011598	Fabp9	2.361743	NM_008003	Fgf15	- 1.758858		
NM_019827	Gsk3b	1.71714	AF197159	Cubn	- 1.5608		
AF374267	Sreb2	2.552183	NM_009995	Cyp21a1	-		
1.971945	AY037763	Pnpla3	1.514965	NM_009230	Soat1	-	
1.770679	NM_022883	Lpin3	1.763003	NM_007856	Dhcr7	-	
1.617239	BC014835	Lbr	1.857061	NM_008291	Hsd17b3	-	
1.527629	NM_011075	Abcb1b	1.533532	NM_008288	Hsd11b1	-	
1.521602	NM_011076	Abcb1a	2.002863	AK018159	Pcsk2	- 1.543528	
1.543528	NM_013518	Fgf9	1.76429	NM_025836	M6prbp1	- 1.65058	
1.65058	AF020738	Fgf12	3.151307	U89431	Pnpla2	- 1.52225	
1.52225	NM_010202	Fgf4	1.674167	Y08566	Lrp2	- 1.566473	
1.566473	NM_016700	Mapk8	2.165714	BC016256	Cyp2d22	- 1.522636	
1.522636	NM_009006	Map4k2	2.145918	NM_009995	Cyp21a1	- 1.971945	
1.971945	AK017631	Nmnat1	1.606443	BC006792	Creb3l2	- 1.555369	
1.555369	NM_009528	Wnt7b	-	2.401384	NM_008160	Gpx1	-
2.401384	NM_008160	Gpx1	-	1.739111			

decline in glycaemia levels after the initial rise at 60 min, whereas acarbose treatment blunted the glucose peak significantly at this time point. These results, along with those of the OGTT, indicate that β -conglutinin may work at the systemic glucose disposal level, with only a minor effect on starch digestion. Related to this, Tachibana et al. showed that soybean β -conglycinin induces higher glucose transporter 4 (GLUT4) membrane translocation in skeletal muscle and increases insulin receptor substrate 2 (IRS-2) in the liver by increasing adiponectin levels in diabetic Goto-Kakizaki rats [36]. These data suggest that this homologous to lupin β -conglutinin protein improves insulin sensitivity. Whether β -conglutinin intake affects the same metabolic processes in these tissues remains to be elucidated.

On the other hand, some authors propose that β -conglutinin peptides produced during *in vitro* digestion may influence glucose metabolism through DPP-IV inhibition. After meal consumption, incretin hormones are released into the bloodstream from the enteroendocrine intestinal cells, inducing 60–70 % of the insulin secretion in healthy subjects [37]. The DPP-IV enzyme rapidly degrades incretins, and therefore DPP-IV inhibition is being exploited to sustain insulin secretion and reduce glycaemia levels [38]. Studies performed by Lammi et al. reported an inhibitory effect of a β -conglutinin-derived peptide on DPP-IV activity using *in vitro* and *ex vivo* models [15,16]. DPP-IV inhibition by this peptide could imply a beneficial influence on postprandial glucose by increasing incretin hormones' half-life and insulin secretion. Paradoxically, however, Schopen et al. found that a meal containing lupin flour reduced healthy subjects' insulinaemic response. The authors

evaluated the influence of a meal including lupin flour or whey protein on postprandial glycaemia and insulinaemia, and although both meals reduced the blood glucose AUC significantly compared to a reference meal, their insulinaemic response was different. Whereas the whey protein meal induced higher insulin levels, the lupin meal decreased them by 39 % during the first 60 min (measured as AUC) [39]. Therefore, future studies should address the effect of lupins and its derived products or constituents on insulin and incretin hormones.

Considering our results shown here, scientific data collected from basic and clinical research highlight the relevance of achieving appropriately controlled postprandial glycaemia. Acarbose is an anti-hyperglycaemic pseudocarbhydrate that can be used to explain this. This drug slows down the digestion of complex carbohydrates by inhibiting α -glucosidase and α -amylase activity [40]. In experimental animals, the reduction of postprandial blood glucose by acarbose treatment has been shown to prevent renal, neurological, or retinal diabetes complications [41]. Moreover, epidemiological studies have shown a relationship between postprandial glucose and cardiovascular disease [42]. Thus, it is now recognized that postprandial hyperglycaemia plays a pivotal role in the development of endothelial dysfunction, oxidative stress, and inflammation, resulting in a deleterious scenario that may progress into cardiovascular events [43]. Therefore, given the pathogenic implications of postprandial hyperglycaemia, our data support the potential use of β -conglutinin in attenuating and controlling glycaemia elevations, but whether this legume protein prevents or ameliorates other physiological disturbances needs to be addressed in additional studies.

We also evaluated the chronic effect of β -conglutinin on some biochemical analytes in animals induced to diabetes by STZ. Although fasting serum glucose concentration did not decrease with the chronic administration of β -conglutinin, the glycaemia levels did not worsen as observed in STZ-Ctrl rats. As expected, the insulin-sensitizing drug rosiglitazone lowered fasting blood glucose levels significantly. Besides, serum triglyceride levels decreased, whereas total serum cholesterol increased. El-Batran et al. reported that rosiglitazone lowered cholesterol and triglyceride levels in rats induced to diabetes by STZ [44], our results, however, agree with those observed in patients treated with rosiglitazone [45]. Nonetheless, our findings should be interpreted with caution since the effects of rosiglitazone are dose-dependent [46].

It is noteworthy to point out that β -conglutinin decreased total cholesterol levels in STZ induced-to-diabetes animals and agree with those obtained by other authors. Sirtori et al. found that administration of *L. albus* protein extract lowered plasma total cholesterol, VLDL- plus LDL-cholesterol, and triglyceride concentrations in rats fed a hypercholesterolaemic diet [47]. Furthermore, Bettzieche et al. reported that *L. albus* protein, compared to casein, decreased LDL-cholesterol but increased cholesterol content in the liver of non-fasted rats fed a hypercholesterolaemic diet [48]. Besides, the inclusion of lupin protein or whole lupin seeds in a hypercholesterolaemic hamster diet resulted in lower plasma total cholesterol concentration [49]. Thus, our results agree with most

previous studies showing a beneficial modulation of circulating cholesterol levels. A higher cholesterol excretion in the faeces of animals fed lupin protein may explain these results [50]. Recently, Naumann et al. systematically studied the influence of diverse lupin constituents on primary bile acids' adsorption. The results revealed that phytochemicals associated with lupin fibre and proteins, such as apigenin derivatives, mediate hydrophobic interactions with bile acids and could exert systemic effects on cholesterol metabolism [51]. Although we did not investigate the presence of phytochemicals in the β -conglutin fraction, its presence cannot be ruled out and needs further investigation.

To better understand the possible molecular targets and mechanisms involved in the biological response to β -conglutin, we performed a liver transcriptome analysis in STZ diabetes-induced rats treated with the protein. Even though our microarray results cannot be considered conclusive, they are useful for proposing possible molecular targets and pathways involved in the observed *in vivo* effects, requiring subsequent studies for clarification.

Relevant to glucose homeostasis, our data showed a down-regulation of Jun kinase activity by β -conglutin treatment. This pathway plays a pathophysiological role in obesity, inflammation, insulin resistance, and diabetes [52]. It is known that the oxidative stress elicited by hyperglycaemia causes deleterious effects on cell physiology and activation of the Jun kinase pathway, promoting pro-inflammatory cascades and insulin resistance that may ultimately lead to the loss of insulin-producing β -cells and dysfunction of insulin-responsive tissues [53]. In accordance with this, our data indicate that β -conglutin down-regulates *Tnf* (tumour necrosis factor- α) gene expression. TNF- α serves as a central cytokine that triggers oxidative stress and inflammation [54]. Related to our findings, Lima-Cabello et al. assessed the effect of five recombinant β -conglutin isoforms (β 1 – 4, and 6) on inflammatory and insulin signalling cascade markers of culture supernatants and peripheral cells from patients with T2D. Interestingly, three of the protein isoforms, as well as a pool prepared with the five of them, promoted

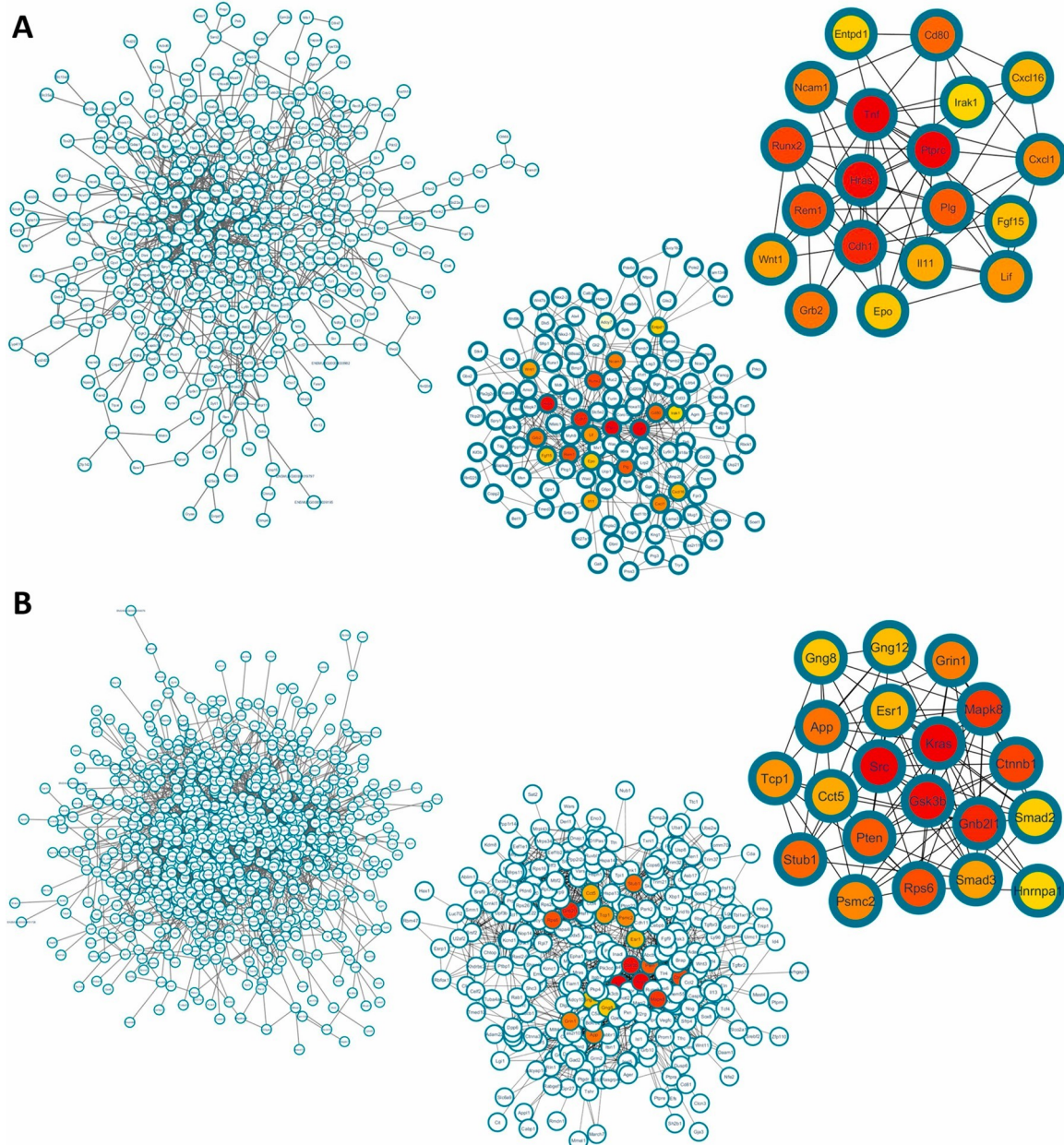


Fig. 9. Identification of hub genes potentially involved in the effects of *L. albus* β -conglutin. The network interaction of differentially expressed genes in the liver of induced-to-diabetes rats treated with β -conglutin was obtained from STRING, and the hub genes among down-regulated (**A**) or up-regulated targets (**B**) were analysed by Cytoscape. Colour intensity of identified hub genes is proportional to the rank based on its interactions by radially (highest ranked genes are depicted in red colour). (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

higher mRNA levels of IRS-1, GLUT4, PKB, and PI3K, whereas lower iNOS and IL-1 β were observed; in contrast, two of the β -conglutin isoforms had a pro-inflammatory effect [13]. More recently, the authors corroborated their findings on exocrine pancreas tumour-derived PANC-1 cells, describing an influence on TNF- α [14]. In support of this, our results indicate that β -conglutin may induce a beneficial response on inflammatory pathways and suggest additional molecular markers that need to be tested. Interestingly, β -conglutin treatment down-regulated glutathione peroxidase 1 gene (*Gpx1*), a target related to oxidative stress and possibly involved in modulation of inflammation cascades as well as the protection against deterioration of fasting glycaemia observed in this study. Thus, additional studies addressing the antioxidant effect of β -conglutin are required to understand its bioactivity in the diabetic state better.

Our microarray experiment results also indicate that the chronic administration of β -conglutin modulated the glycogen synthase kinase β gene (*Gsk3b*). Similarly, in a recent study performed in our laboratory, we identified *Gsk3b* as a target for lupin protein isolate (data submitted for publication). GSK3 β is well recognized as a negative regulator of the insulin signalling cascade and, therefore, a potential therapeutic target for diabetes. GSK3 β is not solely involved in the modulation of glycogen synthesis, but also influences other biological processes such as cell cycle, proliferation, inflammation, and apoptosis [55]. Whether this enzyme is regulated at translational or post-translational levels needs to be evaluated. On the other hand, GSK3 β has also been involved in regulating steroid hormone receptors, including estrogen receptor α (ESR α) encoded by the *Esr1* gene [56].

Accordingly, our microarray data showed up-regulation of the *Esr1* gene, which exhibits potential interactions with molecular targets related to oxidative stress, inflammation, and lipid and carbohydrate metabolism. Although the published results are controversial, phytochemicals such as flavonoids exert bioactivity on ESR α , purportedly producing beneficial health effects [57]. In particular, liver estrogenic activity induced by 17 β -estradiol improves glucose tolerance and insulin sensitivity and reduces gluconeogenesis in mice [58]. Moreover, the role of estrogen in lipid metabolism appears to involve the modulation of sterol regulatory element-binding protein 2 (*Srebp2*) gene expression [59]. The microarray results also showed a positive modulation of *Srebp2* expression. Likewise, Parolini et al. found increased *Srebp2* mRNA levels in animals fed lupin protein and a hypercholesterolaemic diet. In agreement with our results, the authors found lower total cholesterol levels with lupin in the diet [60]. Although our findings corroborate the effect of β -conglutin on this transcription factor, we identified additional targets possibly involved in cholesterol metabolism that need further assessment.

To the best of our knowledge, we report for the first time a positive influence of *L.albus* β -conglutin on carbohydrate tolerance, circulating cholesterol levels in animals induced to diabetes, as well as potential molecular targets and pathways possibly associated with these effects. Nevertheless, we must recognize the limitations present in our study. Here, we focused the experimental design on assessing

glucose homeostasis, and the evaluation of cholesterol lipoproteins was limited. Similarly, we could not evaluate the serum concentration of hormones such as insulin and incretins, which would undoubtedly help to clarify whether β -conglutin effects rely on the modulation of the endocrine function. Therefore, additional studies need to be undertaken to understand further the influence of β -conglutin on serum lipids profile and the endocrine response under abnormal metabolic conditions. Moreover, we assessed the total β -conglutin fraction's bioactivity, but whether the native protein isoforms exert differential effects as indicated by previous reports remains unknown. Finally, although we identified potential molecular targets responsive to β -conglutin treatment through the DNA microarray, complementary methodologies and experimental approaches are necessary to assess this plant protein's influence on aspects related to oxidative stress, inflammation, and estrogenic activity.

5. Conclusions

We found evidence that acute administration of β -conglutin improves the glycaemic response in normal and STZ induced-to-diabetes animals. After seven days, the serum glucose levels of diabetic animals treated with β -conglutin remained stable, while those of the untreated diabetic group increased significantly. On the other hand, the chronic administration of β -conglutin reduced total serum cholesterol levels. Finally, our microarray data contribute to identifying potential molecular targets modulated by β -conglutin and suggest a role for this protein on oxidative stress, inflammation, and estrogenic activity. Future research should focus on evaluating these potential mechanisms.

Funding

The study was funded by Consejo Nacional de Ciencia y Tecnología (CONACyT) through a research grant assigned to C.M.G.-D. (number: 60283). The Universidad de Guadalajara provided partial financial support through grants REC/198/2017, REC/164/2017, and REG/ 0964/19. T.J.G. was the recipient of a graduate scholarship by CONACyT (number: 598227).

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

We thank Dr. Edzard van Santen (Auburn University, Alabama, USA) for the kind donation of certified *Lupinus albus* seeds. We are indebted to Dr. Salvador García for the critical review and suggestions to improve this work. We also thank Lorena Chavez Gonz alez, Sim on Guzm an Le on, and Jorge Ram  rez for technical assistance in the microarray assays.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.biopha.2020.110969>.

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